PLUS AND MINUS STRAINS IN THE GENUS GLOMERELLA

C. W. Edgerton

With one figure in text and plates XXII and XXIII.

Certain species of the Mucorineae, or black mold group of fungi, are composed of two distinct strains, either of which is capable of living by itself and of producing asexual spores, but neither is able alone to produce the zygospores or sexual stage. When the two strains are together, however, and the environment is suitable, the sexual spores are produced abundantly. In the formation of the zygospores, one gamete is produced by one strain and the other gamete by the other strain. In this group of fungi, the strains are very similar; in most cases it being impossible to differentiate between them from a macroscopic or microscopic examination. While these two are without doubt sexual strains, their similarity has made it seem advisable to Blakeslee, who has made a study of many of the forms, to use the indefinite terms, plus and minus, in preference to male and female

Outside of the Mucorineae sexual strains in fungi are either very rare or else have not been recognized and it would seem that they are very rare as many unsuccessful attempts have been made to find them. However in the genus Glomerella, which is the perithecial stage of certain species in the form genera, Gloeosporium and Colletotrichum, a condition exists which approaches that found in the Mucorineae.

The ascogenous stages of many of the Gloeosporiums and Colletotrichums have been known for a number of years. In some of the forms, as for example the one causing the bitter rot disease of apples, the perithecial stage is rather common, while in others its development seems to be rare. Yet with all of the forms the perithecia develop very erratically sometimes present in abundance and other times entirely absent. While working on these fungi during the past several years and trying to find out some of the reasons of the erratic development of the perithecia, the writer has constantly had in mind the possibility of sexual strains. Until about three or four years ago, nothing developed which would in the least show that more than one

strain was present or was necessary for the development of the perithecial stage. Frequently cultures made from single spores would produce the perithecia, this seeming to prove that the species were homothallic, that is, with both sexual organs on the same strain. But during the past four years, several fungi of this group have been found which develop two strains which behave in some ways similar to the strains of the Mucorineae. There is a fertilization between them followed by the development of an abundance of perithecia where they grow together on culture media (plate I, fig. 2). While the behavior of these strains is not identical in all ways with the strains of the black molds, it seems best for lack of better terms to use here also the indefinite terms, plus and minus.

While working on this problem, cultures from several different host plants have been obtained which have shown the presence of the two different sexual strains. The first one that was studied was obtained in northern Louisiana in August, 1910 from the petiole of a leaf of the cottonwood (*Populus deltoides*). This one has been studied for nearly four years. A preliminary report[1] on this was given at the Washington meeting of the Botanical Society of America in December, 1911. This fungus is a typical Gloeosporium and, from a microscopical examination, it is impossible to tell it from *Gloeosporium fructigenum*, the common form causing the bitter rot disease of apples (*Malus sylvestris*). Other hosts from which the two strains have been obtained are the giant beggar weed (*Desmodium tortuosum*), okra (*Hibiscus esculentus*), and morning glory (*Ipomoea purpurea*). Most of the work discussed in this article was carried on with the two forms from cottonwood and morning glory. The cottonwood fungus has been kept in culture for nearly four years and is still producing perithecia in abundance when the two strains are together, and at no time has it shown any signs of deterioration or running out.

The morning glory fungus is also interesting in another way from the fact that only perithecia develop in cultures. While conidia are usually very abundant in Glomerella cultures, none of these have ever been observed in either strain of the anthracnose collected from the morning glory. This is similar to the ascogenous culture from bean (*Phaseolus vulgaris*) which Shear and Wood[2] have reported. There

[1] Edgerton, C. W. Plus and Minus Strains in an Ascomycete. (Abstract) Science, n. s., **35**: 151. 1912.

[2] Shear, C. L., and Wood, Anna K. Studies of Fungous Parasites Belonging to the Genus Glomerella. Bur. Pl. Ind. Bul. **252**: 46–47. 1913.

is no question, however, regarding the identity of this fungus. The perithecia, asci, and ascospores cannot be told in any way from the same structures in other Gloeosporium cultures. Furthermore there were typical conidia along with the perithecia on the morning glory stem from which the cultures were made.

The presence of two strains was first recognized in the culture from the cottonwood. Plantings in petri dishes often showed a colony development like the one illustrated in Plate I, fig. 1. In the center there was a strict growth, black with perithecia, and generally somewhat stellate in shape. Outside of this there was a floccose growth in which perithecia developed in masses in a manner similar to most described Glomerella cultures. Perithecia on the boundary line between these two different growths were always better developed than the perithecia in the black portion of the colony. The plate looked as if there were two fungi present and that the white one was a more rapid grower and had finally outgrown the other and confined it to the central region; and this was finally proven to be the case. Dilution cultures were made from the two portions of the plate and the two distinct forms of growth were isolated. Transfers made from colonies developing from single spores showed two distinct forms and these are the ones which are designated in this paper as the plus and minus strains. These have been studied on various culture media and under various conditions for several years.

The plus strain grows well on all of the ordinary culture media and in most cases it develops an abundant growth of aerial, floccose mycelium of a white or light gray color (upper right and lower left quarters, Plate II, fig. 1). Perithecia usually develop when grown on a good medium such as bean or oat juice agar and they always form in raised masses or nodules similar to the perithecia of other described species of Glomerella. The asci (fig. 1*b*) and ascospores are always well developed in the perithecia. This strain grows more rapidly than the other and when the two are together, it usually confines the latter to the central region of the colony (Plate I, fig. 1).

The minus strain also grows well on most media but produces scarcely any aerial mycelium. The perithecia are produced in great abundance in and on the surface of the culture medium (upper left and lower right quarters, Plate II, fig. 1), generally single though occasionally in twos or threes. The abundance of perithecia generally gives the culture a black color. On ordinary culture media as potato

or bean agar, the perithecia of the forms studied do not mature; they remain small and no asci develop in them. On some special medium such as Clinton's oat juice agar[3] which has been acidified, some of the perithecia come to maturity, but, even on this medium, the asci are usually irregular in shape (fig. 1*a*) and do not seem to have developed properly.

Most of the work in the past on perithecial forms of the genus Glomerella seems to have been done with what is called the plus strain in this article. The description of the genus Glomerella as given by Spaulding and von Schrenk[4] was based on the characters of

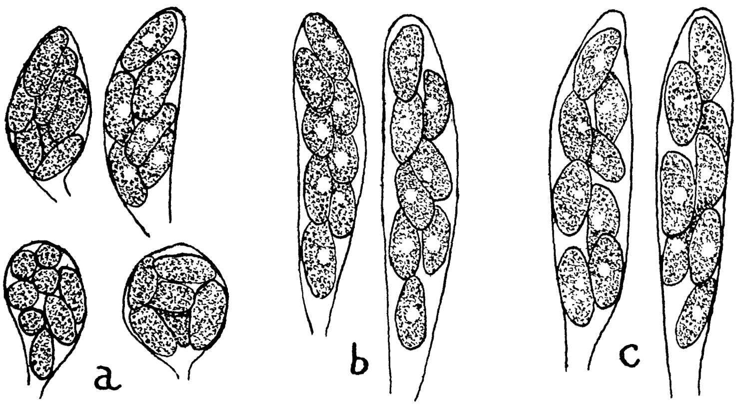


FIG. 1. Asci from the cottonwood Glomerella as formed on oat juice agar: *a*, Asci from the minus strain; *b*, Asci from the plus strain; *c*. Asci from the boundary line between the two strains.

the plus strain of the fungus that causes the bitter rot disease of apples. The cultures with ascogenous stages used by Miss Stoneman,[5] Clinton,[6] Sheldon,[7] and a few others were apparently all of this strain. Yet there are a few cases in the published literature where it seems almost

[3] Clinton, G. P. Oospores of Potato Blight. Conn. Agr. Exp. Sta. Report **1909–1910**: 760–761. 1911.

[4] Von Schrenk, Hermann, and Spaulding, Perley. The Bitter Rot Fungus. Science, n. s., **17**: 750–751. 1903.

[5] Stoneman, Bertha. A Comparative Study of the Development of Some Anthracnoses. Bot. Gaz. **26**: 69–120. 1898.

[6] Clinton, G. P. Apple Rots in Illinois. Ill. Exp. Sta. Bul. **69**. 1902.

[7] Sheldon, J. L. The Ripe Rot or Mummy Disease of Guavas. West Va. Exp. Sta. Bul. **104**. 1906.

certain that the minus strain was also used. In a previous article, the writer[8] described a culture from apple which no doubt belonged to this stage. This culture was explained at the time as a possible mutation but from the observations of the past few years, there seems no doubt but what this was really the minus strain of the bitter rot fungus. Shear[9] also seems to have had both strains of several of the forms on which he has been working though he did not consider them to be sexual strains.

When plantings of the plus and minus strains were made short distances apart on nutrient media in petri dishes and the two developing colonies allowed to grow together, the probability of them being sexual strains became evident. Up to the time the colonies came in contact, the growth of each was similar to that described above. On the boundary line, however, where they were in contact, there developed a black line of perithecia (Plate I, fig. 2). Under suitable temperature and cultural conditions these perithecia from the boundary were mature and shedding their ascospores in four or five days after the strains joined, while the development of the asci in a single strain alone was always much slower. On practically all media tried, especially when acidified, this black line of perithecia developed. On bean agar (Plate I, figs. 2 and 3; Plate II, fig. 1) on which the minus strain produced only immature perithecia, the boundary line was black with perithecia with well-developed asci. On oat juice agar (Plate II, fig. 2) on which the minus strain produced poorly formed asci, the boundary line was a ridge of perithecia sometimes more than a millimeter high with perfectly developed asci. In Plate I, figures 4 and 5, are shown photomicrographs of sections across the boundary line on oat juice agar. On the left side in each case is the minus strain, and on the right the plus strain. In the minus strain may be seen the small immature perithecia. Where the two strains come together, the perithecia are large and well developed and many of them extend up above the culture medium. In Plate I, figure 4, the perithecia on the boundary line are just forming and are still immature while those in figure 5 are well developed and filled with asci. Several hundred of such plate cultures have been made during the past four years and in

[8] Edgerton, C. W. The Physiology and Development of Some Anthracnoses. Bot. Gaz. **45**: 395–396. 1908.

[9] Shear, C. L., and Wood, Anna K. Studies of Fungous Parasites Belonging to the Genus Glomerella. Bur. Pl. Ind. Bul. **252**. 1913.

all cases on a suitable medium this line of perithecia has developed when the plus and minus strains from the same host were used. The four cultures from cottonwood (Plate I, fig. 2), okra (Plate I, fig. 3), morning glory (Plate II, fig. 2), and beggarweed always worked in the same way.

There seemed to be two possible explanations of the formation of the ridge of perithecia on the boundary line between the two strains. It was possible that these two were really different sexual strains of the same fungus or else that the perithecial development was due to some chemical or mechanical stimulus due to the two cultures coming in contact. The latter explanation has been offered by Shear and Wood[10] in their studies on this group. In order to test out this possibility some cultural work was carried on with closely related forms of the genus Glomerella. As is well known, species or races of Gloeosporium and Colletotrichum are very widespread in nature being found on an extremely large number of host plants and many of these are very similar and possibly identical. Cultural work shows the forms to be extremely variable and it is impossible from a morphological study to differentiate between many of them. If the formation of a boundary line of perithecia is due to a chemical or mechanical stimulus, why will not this line develop when one of the strains is grown with other forms? To test this out, the plus and minus strains of the cottonwood and morning glory fungi were grown in the same plate with non-ascogenous cultures of the Gloeosporiums and Colletotrichums isolated from *Malus sylvestris*, *Gossypium hirsutum*, *Capsicum annuum*, *Manihot sp.*, *Melilotus indica*, *Ficus carica*, and *Hibiscus esculentus*. These cultures came in contact with both the plus and minus strains. The plus and minus strains would develop the ridge of perithecia between themselves but in no case would either of the strains develop any perithecia in contact with any of the colonies from the other hosts. Finally to test this out further, another culture was isolated from leaves of *Populus deltoides* in 1912. This proved to be a non-ascogenous culture. This was grown in the same plate with the ascogenous plus and minus strains from the same host but the line of perithecia would not develop between it and either of the strains.

Having been unable to develop a boundary line of perithecia between either the plus or minus strain and any of the other non-

[10] Shear, C. L., and Wood, Anna K. Studies of Fungous Parasites belonging to the Genus Glomerella. Bur. Pl. Ind. Bul. **252**: 74. 1913.

ascogenous anthracnose cultures, an attempt was made to produce this between these strains and ascogenous cultures from other hosts. Plantings were made in the same plate of both of the strains from the cottonwood and also both of the strains from the morning glory so that either strain would come in contact with both of the opposite strains. As the colonies developed, the plus strain of each host came in contact with both of the minus strains. Perithecial ridges developed between the plus and minus strains of the cottonwood fungus and between the plus and minus strains of the morning glory fungus, but no sign of a ridge formed between the plus strain of the cottonwood fungus and the minus strain of the morning glory fungus or between the minus strain of the cottonwood fungus and the plus strain of the morning glory fungus. One of these plates is shown in Plate II, figure 1. The prominent perithecial ridges are between the plus and minus strains of the same host.

These experiments seemed to cast doubt on the possibility of this perithecial development being due to a chemical or mechanical stimulus. It would seem, if this were the cause, that some of these very closely related forms would also have provided this stimulus. These experiments strengthened the theory that the perithecial development was really due to a fertilization between two sexual strains.

To prove that there was really a fertilization between the strains was a rather difficult proposition. The mycelial development is so profuse in the plates that it is impossible to tell by examination whether both strains enter into the formation of the perithecium or not. In order to get some data on this point, an attempt was made to culture the ascospores that developed in a single perithecium on the boundary line. The ascospores of a Glomerella are shed very quickly after they form and they ooze out of the perithecium and remain in a little droplet at the orifice (Plate I, fig. 6). Dilution cultures were made from these little droplets of spores that had oozed out of single perithecia. If there was a cross fertilization, it would be natural to suppose that the spores developing in a single perithecium would develop into two strains while if there were no crossing and the ridge of perithecia on the boundary line was but due to some chemical stimulus, all of the ascospores would develop into but a single strain in the same manner as all of the ascospores from a single perithecium from a single strain do. Plates from nineteen different perithecia were made and of these seventeen showed colonies of both strains. In the other two

only the minus strain seemed to be present though the colonies were so numerous in these that they could not be told with certainty. There was some objection to this method, however, and a slight uncertainty because no matter how careful one might be in transferring from a single droplet of ascospores, there was a slight chance of other loose ascospores being present.

In order to eliminate all of the uncertainty in regard to the strains present in a single perithecium, it was decided to isolate single asci from the boundary line of perithecia and let the different ascospores in them germinate and see if the different ones from a single ascus would develop one or both strains. The isolation of an ascus from a culture of this genus is a difficult operation on account of the fact that the spores are shed as soon as the ascus is mature. In examining a mount made from perithecia, there will be a few scattering asci with mature ascospores present, a great many immature asci generally held in clumps though often loose, and a multitude of loose ascospores. It was found impossible to plate these out dilute enough and then find an ascus in the plate. The only way that seemed possible to isolate the ascus was by some method to pick it up from the mass of spores and transfer it to a marked place in a plate. After many unsuccessful attempts this was found to be possible by attaching a very fine capillary tube which was sealed at the large end to the substage mechanism of the microscope.[11] The capillary tube was firmly held and could be moved in any direction by the various screws which regulate the position of the condenser of the microscope. By means of this capillary tube, the ascus was picked up and then transferred to a sterilized cover slip. The small drop of water on the cover slip was then examined and if only the ascus was found to be present, the cover slip was pushed down into a plate of sterile agar. If other spores were transferred with the ascus, the latter was again picked up and transferred to another cover slip in the same manner or else it was discarded. This was a slow process but it insured the isolation of the ascus. After the ascus was placed in the culture medium, its position was marked on the bottom of the plate with a blue pencil. It was then watched while the spores in it were germinating to make absolutely sure that no other spores were present. The ascospores would germinate in a few hours and finally they would

[11] Edgerton, C. W. A Method of Picking up Single Spores. Phytopathology 4: 115–117. 1914.

develop into a single colony. The presence of one or both strains could usually be told by looking at the colony; but, to make absolutely sure, several times they were reisolated by dilution cultures.

Over forty different asci were transferred, but the ascospores in some failed to germinate and some of the plates were so badly contaminated with bacteria that they had to be discarded. From twenty one colonies that did develop from single asci, twelve contained both the plus and minus strains and nine contained only the minus strain. In Plate II, figure 2, is shown a petri dish in which all of the colonies developed from spores that formed in one of the colonies from a single ascus. Both plus and minus strains are present. This shows that not only were there both strains present in a single perithecium but that they were also present in a single ascus, and this would seem to prove conclusively that there was an actual fertilization between the two strains on the boundary line where they come together in a plate, that one strain furnished the antheridia and the other the oogonia.

All the asci did not produce both the strains but this was hardly to be expected. The culture medium used was oat juice agar, and, as has been noted, some of the perithecia of the minus strain mature on this medium and the asci in these as a rule do not break up as readily as the well developed asci that normally form in the boundary line, and it is possible that some of these might have been transferred. The perithecia of the minus strain develop abundantly all over the surface of the colony and it would be possible to get some of them in a mount made from the boundary. The plus strain only develops in nodules and the chances would not be good for transferring any of these to a mount, and as can be seen there were none of the asci that developed the plus strain alone. Furthermore it is possible that some of the asci that were transferred were not mature. Immature asci are always more abundant in a mount than mature ones on account of the disintegration of the latter at maturity. Whether the immature ascus as a whole would germinate has not been determined, but if it would, the resulting colony would probably be of the minus strain as this is probably the strain that produces the oogonia. And then it is possible that some of the ascospores in an ascus would get started before the others and prevent the latter from developing to any extent. However, the presence of both strains in any of the asci demonstrates the fact that there is really a fertilization between the colonies and that the plus and minus strains should be classified as sexual strains.

The process of fertilization has not been studied to any great extent as yet and it is impossible to explain with certainty the phenomena which have been described in this article. However, a theory that would seem to explain the facts described may be of interest. The minus strain produces an abundance of perithecia and it would seem that this is the strain that produces the oogonia and might be designated as the female strain. As most of the perithecia remain immature or at most but poorly developed, it would seem as if the antheridia in this strain were mostly lacking or poorly developed so that the stimulus following the fertilization is not sufficient to bring the perithecia to maturity. With special stimulating media, the antheridia are possibly better developed or else the stimulating effect of the medium added to that of the fertilization is sufficient to bring some of the asci to maturity. In the plus strain, it is probable that the antheridia are produced very abundantly while the oogonia are produced only sparingly, and where the latter are produced the perithecia form in masses. If the two strains are brought together on the same plate, fertilization takes place and a profuse development of perithecia follows.

It would seem that we have conditions in this group somewhat intermediate between conditions usually found in the Mucorineae. In such fungi as *Rhizopus nigricans*, only one set of the sexual organs is produced on a single strain and the presence of both strains is necessary for the production of the zygospores; while in species like *Sporodinia grandis*, both sexual organs are produced on the same strain. In the Glomerella fungi we have forms in which a fertilization between strains is not necessary for, but stimulates the production of the sexual stage.

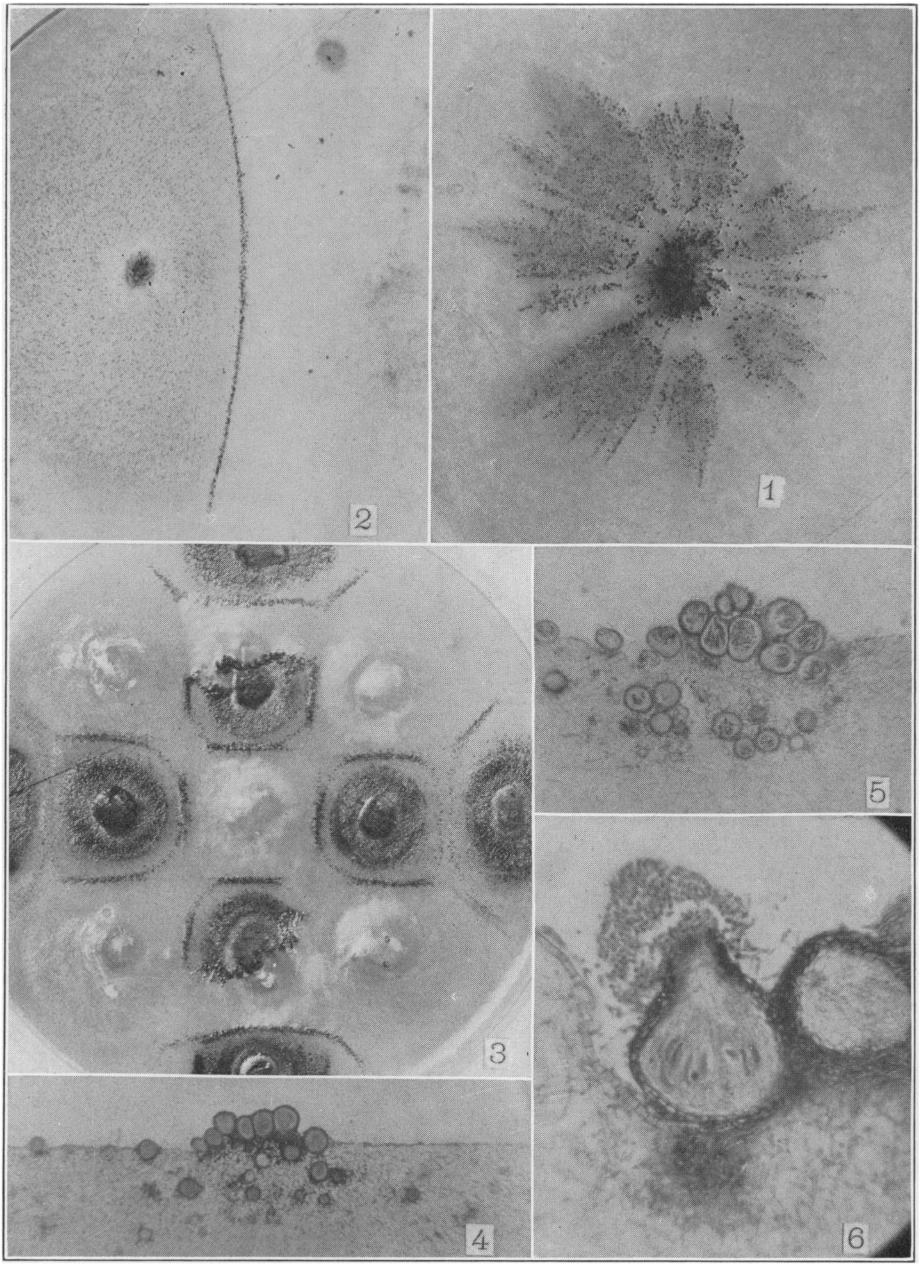
Whether other forms of the ascomycetes may be found in which a fertilization between different strains stimulates the development of the fruit body and ascospores is a question. The Glomerella forms show so much variability that it is possible that we have in the cultures that have been described merely isolated variations that may not be common in the ascomycetes as a group or even within the genus Glomerella itself. Yet the fact that two sexual strains may sometimes be present adds another factor to be considered in all work dealing with the development of ascogenous stages.

LOUISIANA AGRICULTURAL EXPERIMENT STATION,
BATON ROUGE, LA.

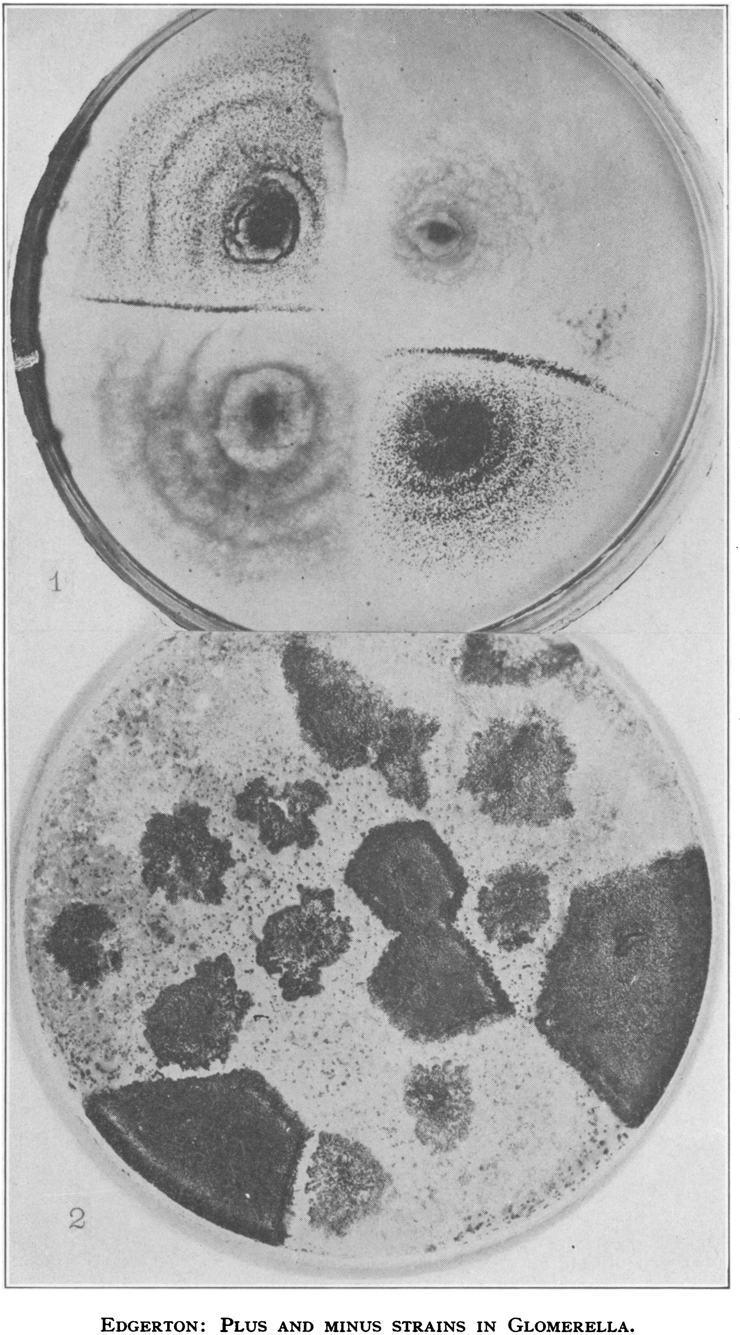
Explanation of Plates XXII and XXIII

Plate XXII. Plus and minus strains of Glomerella. 1. Plus and minus strains of the cottonwood Glomerella in a single colony, showing the minus strain with the numerous perithecia confined in the central portion of the colony with the white plus strain on the outside. 2. Plus (right) and minus (left) strains of the cottonwood fungus with the line of perithecia between them. 3. Plus and minus strains of the Glomerella from okra. 4. Section across boundary line between plus and minus strains; perithecia just forming. 5. Section across boundary line between plus and minus strains; perithecia mature. 6. Perithecium from the boundary line between the plus and minus strains showing the ascospores in a droplet at the orifice.

Plate XXIII. Plus and minus strains of Glomerella. 1. Plus and minus strains of the Glomerellas from cottonwood (left) and morning glory (right) showing the boundary line of perithecia between the two strains from the same host but not between the two strains from different hosts. 2. Plus and minus colonies of the morning glory Glomerella on oat juice agar, isolated from a colony that developed from a single ascus from the boundary line between the two strains.



EDGERTON: PLUS AND MINUS STRAINS IN GLOMERELLA.



EDGERTON: PLUS AND MINUS STRAINS IN GLOMERELLA.